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Terms	Documents
L2 and (antisense or ribozyme$)	16

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Derwent World Patents Index
IBM Technical Disclosure Bulletins

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DATE: Tuesday, April 29, 2003 Printable Copy Create Case

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L3	L2 and (antisense or ribozyme$)	16	L3
L2	(pI3K adj p85) and (hypoglycemi$ or glucose or insulin)	21	L2
L1	(pI3K adj p85) same (hypoglycemi$ or glucose or insulin)	2	L1

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Set	Items	Description
S1	3	(PI3K (W) P85) (S) (ANTISENSE OR RIBOZYME?)
S2	3	RD (unique items)

>>>KWIC option is not available in file(s): 399

2/3,K/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12974392 BIOSIS NO.: 200100181541
***Antisense* inhibition of *PI3K* *p85* expression.**
AUTHOR: Monia Brett P; Cowsert Lex M
JOURNAL: Official Gazette of the United States Patent and Trademark Office
Patents 1237 (2):pNo Pagination Aug. 8, 2000
MEDIUM: e-file
ISSN: 0098-1133
DOCUMENT TYPE: Patent
RECORD TYPE: Abstract
LANGUAGE: English

***Antisense* inhibition of *PI3K* *p85* expression.**

ABSTRACT: Antisense compounds, compositions and methods are provided for modulating the expression of *PI3K* *p85*. The compositions comprise *antisense* compounds, particularly *antisense* oligonucleotides, targeted to nucleic acids encoding *PI3K* *p85*. Methods of using these compounds for modulation of *PI3K* *p85* expression and for treatment of diseases associated with expression of *PI3K* *p85* are provided.

2/3,K/2 (Item 1 from file: 399)
DIALOG(R)File 399:CA SEARCH(R)

136395986 CA: 136(26)395986v PATENT
Antisense modulation of phosphatidylinositol 3 kinase (PI3K) p85 expression for disease treatment
INVENTOR(AUTHOR): Monia, Brett P.; Cowsert, Lex M.; Murray, Susan F.; Butler, Madeline M.; Dean, Nicholas M.
LOCATION: USA
ASSIGNEE: Isis Pharmaceuticals, Inc.
PATENT: PCT International ; WO 200240637 A2 DATE: 20020523
APPLICATION: WO 2001US45006 (20011119) *US 715983 (20001120)
PAGES: 121 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: C12N-000/A
DESIGNATED COUNTRIES: AE; AG; AL; AM; AT; AU; AZ; BA; BB; BG; BR; BY; BZ; CA; CH; CN; CO; CR; CU; CZ; DE; DK; DM; DZ; EC; EE; ES; FI; GB; GD; GE; GH; GM; HR; HU; ID; IL; IN; IS; JP; KE; KG; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MA; MD; MG; MK; MN; MW; MX; MZ; NO; NZ; PL; PT; RO; RU; SD; SE; SG; SI; SK; SL; TJ; TM; TR; TT; TZ; UA; UG; US; UZ; VN; YU; ZA; ZW; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM DESIGNATED REGIONAL: GH; GM; KE; LS; MW; MZ; SD; SL; SZ; TZ; UG; ZM; ZW; AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE; TR; BF; BJ; CF; CG; CI; CM; GA; GN; GQ; GW; ML; MR; NE; SN; TD; TG

2/3,K/3 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0297722 DBR Accession No.: 2002-19569 PATENT

***Antisense* compounds targeted against polynucleotides encoding *PI3K* *p85* useful for treating e.g. cancer, Type 2 diabetes, obesity - for use in cancer, diabetes, obesity and inflammation diagnosis, prevention and therapy**

AUTHOR: MONIA B P; COWSERT L M, MURRAY S F; BUTLER M M; DEAN N M

PATENT ASSIGNEE: ISIS PHARM INC 2002
PATENT NUMBER: WO 200240637 PATENT DATE: 20020523 WPI ACCESSION NO.: 2002-519374 (200255)
PRIORITY APPLIC. NO.: US 715983 APPLIC. DATE: 20001120
NATIONAL APPLIC. NO.: WO 2001US45006 APPLIC. DATE: 20011119
LANGUAGE: English

***Antisense* compounds targeted against polynucleotides encoding *PI3K* *p85* useful for treating e.g. cancer, Type 2 diabetes, obesity - for use in cancer, diabetes, obesity and inflammation diagnosis, prevention and therapy**

ABSTRACT: DERWENT ABSTRACT: NOVELTY - An compound (I) 8-30 nucleobases in length targeted to a nucleic acid molecule encoding *PI3K* *p85* (II), where (I) specifically hybridizes with and inhibits the expression of PI3K p85a, and optionally alters the ratio of PI3K p85a to PI3K p5a expressed by a cell or tissue, is new. WIDER DISCLOSURE - Chimeric compounds comprising (I) are also disclosed. BIOTECHNOLOGY - Preferred Compound: (I) Is an *antisense* oligonucleotide, and is preferably targeted to a region of f nucleotide sequence encoding PI3K p85a, which is not found in a polynucleotide encoding PI3K p50a, and further inhibits the expression of all splice variants encoded by PI3K p85a, where the *antisense* compound alters the ratio of PI3K p85a to PI3K p50a expressed by a cell or tissue. ACTIVITY - Cytostatic; Antidiabetic; Anorectic; Antitumor; Antiinflammatory. MECHANISM OF ACTION...

... of (II) expression (claimed). (I) (comprising 36 defined sequences as given in the specification) was tested for its (II) expression inhibitor activity. The results showed *antisense* oligonucleotides atttcctgggatgtgcg, ccgctcttgggtctggca, tcaacttcttttgccgaa, ttgcccaaccac tcgttc, ctttgtttcggttgctgc, ctttacttcgccgtccac, ccaggctaaaccaggctg and tgtctgggtaccgtg exhibited at least 30% inhibition of (II) expression. USE - (I) is useful for decreasing blood...

...to PI3K 50a/50a in human cell or tissues; or for treating a human having a disease or condition associated with PI3K signal transduction or *PI3K* *p85* expression (claimed). *Antisense* compounds are commonly used as research reagents and diagnostics. *Antisense* compounds either alone or in combination with other *antisense* compounds or therapeutics can be used as tools in differential and/or combinatorial analyses to elucidate expression patterns of a portion or the entire complement of genes expressed within cells and tissues. Use of the *antisense* compounds and the above may also be useful prophylactically, e.g., to prevent or delay infection, inflammation or tumor formation. ADMINISTRATION - (I) Is administered by...

... by inhalation or insufflation of powders or aerosols, including by nebulizer, intratracheal, intranasal, epidermal and transdermal, oral or parenteral. No specific dosage is given. EXAMPLE - *Antisense* compound that inhibits *PI3K* *p85* was synthesized by standard solid phase synthesis. (121 pages)

DESCRIPTORS: human recombinant *PI3K* *p85* protein prep., isol., *antisense*, appl. cancer, diabetes, obesity, inflammation diagnosis, prevention, therapy animal mammal tumor DNA sequence protein sequence (21, 51)

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Set	Items	Description
S1	3	(PI3K (W) P85) (S) (ANTISENSE OR RIBOZYME?)
S2	2	RD (unique items)
S3	29	(PI3K (W) P85) AND (DIABET? OR GLUCOSE OR INSULIN)
S4	16	RD (unique items)

>>>KWIC option is not available in file(s) 399

4/3,K/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

14175749 BIOSIS NO.: 200300169778
Altered signaling and cell cycle regulation in embryonal stem cells with a disruption of the gene for phosphoinositide 3-kinase regulatory subunit p85alpha.
AUTHOR: Hallmann Daniel; Truemper Katja; Truesheim Heidi; Ueki Kohjiro; Kahn C Ronald; Cantley Lewis C; Fruman David A; Hoersch Dieter(a)
AUTHOR ADDRESS: (a)Dept of Internal Medicine, Division of Gastroenterology and Metabolism, Philipps-University, Baldingerstrasse, D-35033, Marburg, Germany**Germany E-Mail: hoerschd@post.med.uni-marburg.de
JOURNAL: Journal of Biological Chemistry 278 (7):p5099-5108 February 14 2003 2003
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

...ABSTRACT: derived from the Pik3r1 gene, which also yields alternatively spliced variants p50alpha and p55alpha. It has been proposed that excess monomeric p85 competes with functional *PI3K* *p85*-p110 heterodimers. We examined embryonic stem (ES) cells with heterozygous and homozygous disruptions in the Pik3r gene and found that wild type ES cells express virtually no monomeric p85alpha. Although, IGF-1-stimulated PI3K activity associated with *insulin* receptor substrates was unaltered in all cell lines, p85alpha-null ES cells showed diminished protein kinase B activation despite increased PI3K activity associated with the...
...REGISTRY NUMBERS: *INSULIN*-LIKE GROWTH FACTOR-1
DESCRIPTORS
CHEMICALS & BIOCHEMICALS: ...*insulin* receptor substrates...

...*insulin*-like growth factor-1

4/3,K/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13871050 BIOSIS NO.: 200200498871
PI 3-kinase and its up- and down-stream modulators as potential targets for the treatment of type II *diabetes*.
AUTHOR: Jiang Guoqiang(a); Zhang Bei B
AUTHOR ADDRESS: (a)Metabolic Disorders-Diabetes, Merck Research Laboratories, RY80N-C31, P.O. Box 2000, Rahway, NJ, 07065**USA E-Mail: guoqiangjiang@merck.com
JOURNAL: Frontiers in Bioscience 7 Cited May 17, 2002 :pd903-917 April 1, 2002
MEDIUM: online

ISSN: 1093-4715
DOCUMENT TYPE: Literature Review
RECORD TYPE: Abstract
LANGUAGE: English

PI 3-kinase and its up- and down-stream modulators as potential targets for the treatment of type II *diabetes*.

ABSTRACT: Type 2 *diabetes* is caused by a combination of impaired *insulin* secretion and, to a greater extent, resistance of target tissues to *insulin* action. Phosphoinositide 3-kinase (PI3K) plays a key role in *insulin* signaling and has been shown to be blunted in tissues of type 2 *diabetes* subjects. There is emerging biochemical and, particularly, genetic evidence suggesting that *insulin* resistance can potentially be treated via modulation of PI3K by targeting PI3K itself or its up and down-stream modulators. These potential targets include Src...

...2 domain containing inositol 5-phosphatase 2 (SHIP2), phosphatase and tensin homolog deleted on chromosome ten (PTEN), IkappaB kinase beta (IKKbeta), PKC isoforms, and the *PI3K* *p85* subunit. There is evidence suggesting that their inhibition affects PI3K activity and improves *insulin* sensitivity in vivo. In the current review, we will discuss the role of these molecules in *insulin*-mediated activation of PI3K, the rational for targeting these molecules for *diabetes* treatment, and some critical issues in terms of drug development.

DESCRIPTORS:
DISEASES: type II *diabetes* {non-*insulin*-dependent *diabetes* mellitus ..
ALTERNATE INDEXING: *Diabetes* Mellitus, Non-*Insulin*-Dependent (MeSH)

4/3,K/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13855806 BIOSIS NO.: 200200484627
ETV6-NTRK3 transformation requires *insulin*-like growth factor 1 receptor signaling and is associated with constitutive IRS-1 tyrosine phosphorylation.

AUTHOR: Morrison Kevin B; Tognon Cristina E; Garnett Mathew J; Deal Cheri; Sorensen Poul H B(a)
AUTHOR ADDRESS: (a)BC Research Institute for Children's and Women's Health, West 28th Ave., Room 3082-950, Vancouver, BC, V5Z 4H4**Canada E-Mail: psor@interchange.ubc.ca
JOURNAL: Oncogene 21 (37):p5684-5695 22 August, 2002
MEDIUM: print
ISSN: 0950-9232
DOCUMENT TYPE Article
RECORD TYPE: Abstract
LANGUAGE: English

ETV6-NTRK3 transformation requires *insulin*-like growth factor 1 receptor signaling and is associated with constitutive IRS-1 tyrosine phosphorylation.

.. ABSTRACT: pathway. However, the role of trisomy 11 in CFS and CMN remains unknown. In this study we demonstrate elevated expression of the chromosome 11p15.5 *insulin*-like growth factor 2 gene (IGF2) in CFS and CMN tumors. Moreover, we present evidence that an intact IGF signaling axis is essential for in...

.. IGFRI), but transformation activity was fully restored in R -cells engineered to re-express IGFRI (R+ cells). We also observed that the major IGFRI substrate, *insulin*-receptor substrate-1 (IRS-1), was constitutively tyrosine phosphorylated and could be co-immunoprecipitated with EN in either R- or R+ cells expressing the EN oncoprotein. IRS-1 association with Grb2 and *PI3K* *p85*, which link IGFRI to the Ras-MAPK

and PI3K-Akt pathways, respectively, was enhanced in both cell types in
the presence of EN. However, activation ...
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ...*insulin*-like growth factor 1 receptor...

...*insulin*-receptor substrate-1
.. GENE NAME: human IGF2 gene (human *insulin*-like growth factor 2 gene)
Hominidae)

4/3,K/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

13701559 BIOSIS NO.: 200200330381
The phosphatidylinositol 3-kinase pathway is critical for the regulation of branched-chain ketoacid dehydrogenase activity by glucocorticoids.
AUTHOR: Wang X(a); Du J(a); Price S R(a)
AUTHOR ADDRESS: (a Renal Division, Emory University, Atlanta, GA**USA
JOURNAL: Journal of the American Society of Nephrology 12 (Program and Abstract Issue):p829A September, 2001
MEDIUM: print
CONFERENCE/MEETING: ASN (American Society of Nephrology)/ISN (International Society of Nephrology) World Congress of Nephrology San Francisco, CA, USA October 10-17, 2001
ISSN 1046-6673
RECORD TYPE: Citation
LANGUAGE: English

...REGISTRY NUMBERS: *INSULIN*;
DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: IRS-1 {*insulin* receptor substrate-1...

...*insulin*; ...
...phosphatidylinositol 3-kinase p85 regulatory subunit {*PI3K* *p85* regulatory subunit
GENE NAME: pig *PI3K* *p85* gene (pig phosphatidylinositol 3-kinase p85 gene) (Suidae...

4/3,K/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10106604 BIOSIS NO.: 199698561522
Compensatory alterations for *insulin* signal transduction and *glucose* transport in *insulin*-resistant *diabetes*.
AUTHOR: Bonini James A(a); Colca Jerry R; Dailey Charlene; White Morris; Hoffman Cecilia
AUTHOR ADDRESS: (a)Howard Hughes Medical Inst., 5841 South Maryland Ave., Chicago, IL 60637**USA
JOURNAL American Journal of Physiology 269 (4 PART 1):pE759-E765 1995
ISSN 0002-9513
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE English

Compensatory alterations for *insulin* signal transduction and *glucose* transport in *insulin*-resistant *diabetes*.

ABSTRACT: *Insulin* binding activates the receptor tyrosine kinase toward the *insulin* receptor substrate-1 (IRS-1). Phosphorylated IRS-1 then interacts with the p85-alpha subunit of phosphatidylinositol 3-kinase (PI3K), Nck, growth factor receptor-bound protein 2 (GRB2), and Syp, thus branching *insulin*'s signal for both mitogenic and metabolic responses. To determine whether the expression of these proteins is altered in *insulin* resistance, the levels of these proteins were compared in

adipose and liver tissues of nondiabetic mice and obese *insulin* -resistant *diabetic* KKA-y mice. IR and *PI3K* *p85*-alpha protein levels were significantly lower in KKA-y mice than in control nondiabetic mice, whereas IRS-1 protein levels were not altered. In contrast, the protein levels of GRB2, Nck, Syp, and GLUT-1 were dramatically elevated in KKA-y fat, with less striking changes in liver. Treatment of *diabetic* animals with pioglitazone, an *insulin*-sensitizing antihyperglycemic agent, partially corrected the expression of some of these proteins. Taken together, these findings suggest that the *insulin* -resistant *diabetic* condition is characterized by changes in expression of *insulin* signal transduction components that may be associated with altered *glucose* metabolism.

...REGISTRY NUMBERS: *INSULIN*; ...

...*GLUCOSE*;

DESCRIPTORS:

CHEMICALS & BIOCHEMICALS *INSULIN*; *GLUCOSE*;

4/3,K/6 (Item 1 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

06839590 Genuine Article#: ZV966 No. References: 61

Title: Phosphorylation of the Grb2- and phosphatidylinositol 3-kinase p85-binding p36/38 by Syk in Lck-negative T cells

Author(s): vonWillebrand M; Williams S; Tailor P; Mustelin T (REPRINT)
Corporate Source: LA JOLLA INST ALLERGY & IMMUNOL,DIV CELL BIOL, 10355 SCI CTR DR/SAN DIEGO//CA/92121 (REPRINT); LA JOLLA INST ALLERGY & IMMUNOL,DIV CELL BIOL/SAN DIEGO//CA/92121

Journal: CELLULAR SIGNALLING, 1998, V10, N6 (JUN), P407-413
ISSN: 0898-6568 Publication date: 19980600
Publisher: ELSEVIER SCIENCE INC, 655 AVENUE OF THE AMERICAS, NEW YORK, NY 10010
Language: English Document Type: ARTICLE (ABSTRACT AVAILABLE)

...Abstract: the same time, expression of Syk resulted in the activation-dependent phosphorylation of three proteins that bound to the src homology 2 (SH2) domains of *PI3K* *p85*. The strongest of these bands had an apparent molecular mass of 36-38 kDa on SDS gels, and it was quantitatively removed from the lysates ...

...Identifiers--PROTEIN-KINASE-C; RECEPTOR TYROSINE KINASES; ANTIGEN RECEPTOR; SIGNAL-TRANSDUCTION; *INSULIN* STIMULATION; CATALYTIC SUBUNIT; DEPENDENT PATHWAY; SH2 DOMAINS; ACTIVATION; RAF-1

4/3,K/7 (Item 2 from file: 34)

DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05994064 Genuine Article#: XM783 No. References: 67

Title: Requirement of phosphatidylinositol 3-kinase-dependent pathway and Src for Gas6-Axl mitogenic and survival activities in NIH 3T3 fibroblasts

Author(s): Goruppi S; Ruaro E; Varnum B; Schneider C (REPRINT)
Corporate Source: LNCIB,AREA SCI PK, PADRICIANO 99/I-34012 TRIESTE//ITALY/ (REPRINT); LNCIB,AREA SCI PK/I-34012 TRIESTE//ITALY/; INT CTR GENET ENGN & BIOTECHNOL,/TRIESTE//ITALY/; UNIV UDINE,DIPARTIMENTO SCI & TECHNOL BIOMED/I-33100 UDINE,/ITALY/; AMGEN CORP,/THOUSAND OAKS//CA/91320

Journal: MOLECULAR AND CELLULAR BIOLOGY, 1997, V17, N8 (AUG), P4442-4453
ISSN: 0270-7306 Publication date: 19970800
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171
Language: English Document Type: ARTICLE (ABSTRACT AVAILABLE)

...Abstract: of Gas6 requires phosphatidylinositol 3-kinase (PI3K) activity

since it is abrogated both by the specific inhibitor wortmannin and by overexpression of the dominant negative *PI3K* *p85* subunit, Consistently, Gas6 activates the PI3K downstream targets S6K and Akt, whose activation is abrogated by addition of wortmannin. Moreover, rapamycin treatment blocks Gas6-induced...

Research Fronts: 95-1162 004 (PHOSPHATIDYLINOSITOL 3-KINASE; RAS-INDEPENDENT *INSULIN* SIGNALING PATHWAYS; EPIDERMAL GROWTH-FACTOR RECEPTOR; TYROSINE PHOSPHORYLATION; SHC PROTEINS)
95-4290 001 (N-TERMINAL SH3 DOMAIN; PROTEIN PRODUCT OF THE C-CBL PROTOONCOGENE; TYROSINE...

4/3,K/8 (Item 1 from file: 370)

DIALOG(R)File 370:Science

00509614 (USE 9 FOR FULLTEXT)

Xid-Like Immunodeficiency in Mice with Disruption of the p85a Subunit of Phosphoinositide 3-Kinase

Suzuki, Harumi; Terauchi, Yasuo; Fujiwara, Mari; Aizawa, Shinichi; Yazaki, Yoshio; Kadowaki, Takashi; Koyasu, Shigeo

H. Suzuki, M. Fujiwara, S. Koyasu, Department of Immunology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. Y. Terauchi, Y. Yazaki, T. Kadowaki, Department of Internal Medicine, University of Tokyo, Graduate School of Medicine, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan. S. Aizawa, Department of Morphogenesis, Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine, Kumamoto 860, Japan.

Science Vol. 283 5400 pp. 390

Publication Date: 1-15-1999 (990115) Publication Year: 1999

Document Type: Journal ISSN: 0036-8075

Language: English

Section Heading: Research Articles

Word Count: 1578

(THIS IS THE FULLTEXT)

...Text: gene encodes two additional minor alternative splicing isoforms, p55a and p50a (B3) (B4) . Binding of p85a to tyrosine-phosphorylated proteins such as IRS-1 in *insulin* signaling (B5) and CD19 in B cell antigen-receptor signaling (B6) activates PI3K activity of the p110 subunit. To elucidate precise roles of p85a in...The p85a.sup(-/-) mice still expressed minor regulatory subunits of *PI3K* (*p85* (beta) , p55 (gamma) , p50a, and p55a) in various tissues. Targeted disruption of all isoforms derived from the p85a gene also caused immune-deficient phenotypes nearly...

4/3,K/9 (Item 1 from file: 399)

DIALOG(R)File 399:CA SEARCH(R)

136395986 CA: 136(26)395986v PATENT

Antisense modulation of phosphatidylinositol 3 kinase (PI3K) p85 expression for disease treatment

INVENTOR(AUTHOR): Monia, Brett P.; Cowsert, Lex M.; Murray, Susan F.; Butler, Madeline M.; Dean, Nicholas M.

LOCATION: USA

ASSIGNEE: Isis Pharmaceuticals, Inc.

PATENT: PCT International ; WO 200240637 A2 DATE: 20020523

APPLICATION: WO 2001US45006 (20011119) *US 715983 (20001120)

PAGES: 121 pp. CODEN: PIXXD2 LANGUAGE: English CLASS: C12N-000/A

DESIGNATED COUNTRIES: AE; AG; AL; AM; AT; AU; AZ; BA; BB; BG; BR; BY; BZ; CA; CH; CN; CO; CR; CU; CZ; DE; DK; DM; DZ; EC; EE; ES; FI; GB; GD; GE; GH; GM; HR; HU; ID; IL; IN; IS; JP; KE; KG; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MA; MD; MG; MK; MN; MW; MX; MZ; NO; NZ; PL; PT; RO; RU; SD; SE; SG; SI; SK; SL; TJ; TM; TR; TT; TZ; UA; UG; US; UZ; VN; YU; ZA; ZW; AM; AZ; BY; KG; KZ; MD; RU; TJ; TM DESIGNATED REGIONAL: GH; GM; KE; LS; MW; MZ; SD; SL; SZ

; TZ; UG; ZM; ZW; AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LU; MC; NL; PT; SE; TR; BF; BJ; CF; CG; CI; CM; GA; GN; GQ; GW; ML; MR; NE; SN; TD; TG

4/3,K/10 (Item 1 from file: 135)
DIALOG(R)File 135:NewsRx Weekly Reports

0000070598 (USE FORMAT 7 OR 9 FOR FULLTEXT)
Lower *insulin* sensitivity found in Mexican Americans
Diabetes Week, December 2, 2002, p.13

DOCUMENT TYPE: Expanded Reporting LANGUAGE: English
RECORD TYPE: FULLTEXT
WORD COUNT: 948

Lower *insulin* sensitivity found in Mexican Americans

...TEXT: delivery of primary care services, but for the future services that will be necessary to diagnose and treat a population highly susceptible to type 2 *diabetes*.

Mexican Americans are diagnosed with type 2 *diabetes* mellitus (T2DM) two to three times more frequently than are non-Hispanic white (NHW) Americans. Scientists have found that *insulin* resistance is key in the cause for the disease; studies have revealed that Mexican Americans of both genders and all ages demonstrate greater levels of *insulin* resistance when compared with the NHW population.

Although the reasons are unclear, some suggest that genetic factors could explain the higher prevalence of *insulin* resistance in this Hispanic population group, considering that 35% of their genetic Mexican American make-up is attributable to Native American ancestry.

It is also possible that lifestyle factors, including diet and exercise, contribute to the ethnic differences in *insulin* resistance. Visceral adiposity (obesity), exercise, and dietary fat have all been shown to impact peripheral *insulin* resistance.

A new research study that compares behavioral, metabolic, and molecular behavior between Mexican Americans and non-Hispanic whites offers new clues on why public...

...dietary needs of our citizens and residents from the south (Ho RC, Davy KP, Hickey MS, et al., Behavioral, metabolic, and molecular correlates of lower *insulin* sensitivity in Mexican Americans. American Journal of Physiology-Endocrinology and Metabolism, 2002;283(4):E799-808).

In previous studies, Mexican Americans have been shown to exhibit lower *insulin* sensitivity independently of body fat and body fat patterning. However, this issue is not entirely resolved given that studies that have documented diminished *insulin* sensitivity in nonobese, nondiabetic Mexican Americans compared with NHW used less sensitive methods to estimated Mexican American body fat and obesity. Furthermore, the possible contribution...
...central adiposity, are less physically active, and consume a more atherogenic diet, it is important to examine these factors as possible contributors to the lower *insulin* sensitivity in Mexican Americans.

This study had two specific aims: First, to determine whether differences in *insulin* sensitivity persist between these two groups after controlling for the effects of acute and chronic exercise, abdominal fat distribution, and dietary intake. Second, to ascertain whether Mexican Americans exhibit lower skeletal muscle protein concentrations of IR, *PI3K* *p85*, Akt1, Akt2, and GLUT4 compared with NHW after controlling for these same potential confounders.

Thirteen nonobese Mexican Americans (7 females, 6 males) were matched to...

...characteristics: being nonsmoking, apparently healthy individuals with no overt signs or symptoms of disease as determined by a medical history, and having normal fasting blood *glucose*, no past or present history of

endocrine disorders, and resting blood pressure of 110/90 mm Hg. To be appropriately identified as Mexican Americans, each...

...four grandparents. The Colorado State University Human Research Committee approved the study protocol.

The researchers found that: * Mexican Americans were found to be significantly less *insulin* sensitive compared with their NHW counterparts. * There were no significant differences between the two groups with regard to skeletal muscle protein abundance of IR, *PI3K* *p85* , Akt1, Akt2, or GLUT4. Skeletal muscle protein abundance of IR was significantly associated with fasting plasma *insulin*. * Percent total energy intake from palmitoleic acid was significantly higher among Mexican Americans, with a trend toward higher percent total energy intake from palmitic acid...

...acid and lower fiber intake among Mexican Americans.

There are three major significant findings in this study: * First, nonobese, nondiabetic Mexican Americans adults were less *insulin* sensitive compared with NHW adults, even when the potential roles of cardiorespiratory fitness, acute exercise, and total and regional adiposity were accounted for. * Second, skeletal muscle protein abundance of IR, *PI3K* *p85*, Akt1, Akt2, and GLUT4 was not significantly different between the two groups and therefore does not account for the group differences in *insulin* sensitivity. * Finally, group differences in *insulin* sensitivity were attenuated to losing statistical significance after dietary intakes of palmitic acid, palmitoleic acid or skeletal muscle IR protein content were accounted for.

This study demonstrates that lower *insulin* sensitivity persists in nonobese, nondiabetic Mexican Americans compared with their non-Hispanic white counterparts, even after acute and chronic effects of exercise and abdominal fat distribution are accounted for. Furthermore, protein abundance of skeletal muscle IR, *PI3K* *p85*, Akt1, Akt2, or GLUT4 does not explain these differences. Differences in *insulin* sensitivity are lost when dietary intakes of palmitate and palmitoleate are accounted for, suggesting the possibility that these factors may contribute to the lower *insulin* sensitivity seen in Mexican Americans.

This article was prepared by *Diabetes* Week editors from staff and other reports.

DESCRIPTORS: *Diabetes*; Endocrinology; All News; Professional News
SUBJECT HEADING: Type 2 *Diabetes*

4/3,K/11 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0297722 DBR Accession No.: 2002-19569 PATENT
Antisense compounds targeted against polynucleotides encoding *PI3K* *p85* useful for treating e.g. cancer, Type 2 *diabetes*, obesity - for use in cancer, *diabetes*, obesity and inflammation diagnosis, prevention and therapy
AUTHOR: MONIA B P; COWSERT L M; MURRAY S F; BUTLER M M; DEAN N M
PATENT ASSIGNEE: ISIS PHARM INC 2002
PATENT NUMBER: WO 200241637 PATENT DATE: 20020523 WPI ACCESSION NO.: 2002-519374 (200255)
PRIORITY APPLIC. NO.: US 715983 APPLIC. DATE: 20001120
NATIONAL APPLIC. NO.: WO 2001US45006 APPLIC. DATE: 20011119
LANGUAGE: English

Antisense compounds targeted against polynucleotides encoding *PI3K* *p85* useful for treating e.g. cancer, Type 2 *diabetes*, obesity - for use in cancer, *diabetes*, obesity and inflammation diagnosis, prevention and therapy
ABSTRACT: DERWENT ABSTRACT: NOVELTY - An compound (I) 8-30 nucleobases in length targeted to a nucleic acid molecule encoding *PI3K* *p85* (II), where (I) specifically hybridizes with and inhibits the expression of

PI3K p85a, and optionally alters the ratio of PI3K p85a to PI3K p5a expressed...

...oligonucleotides atttcctgggatgtgcg, ccgctcttgggtctggca, tcaacttcttttgccg aa, ttgcccaaccactcgttc, ctttgtttcggttgctgc, ctttacttcgccgtccac, ccaggctaaaccaggctg and tgtctgggtaccgtg exhibited at least 30% inhibition of (II) expression. USE - (I) is useful for decreasing blood *glucose* or *insulin* levels, or preventing or delaying the onset of an increase in blood *glucose* or *insulin* levels in an animal preferably a human or a rodent, which is *diabetic*; preventing or delaying the onset of f disease or condition associated with (II) in an animal preferably human, where the disease condition is a metabolic disease or condition which is *diabetes* especially Type 2 *diabetes*, obesity or a hyperproliferative condition which is cancer;modulating PI3K signal transduction in cell or tissues; altering the ratio of PI3K p85a to PI3K 50a/50a in human cell or tissues; or for treating a human having a disease or condition associated with PI3K signal transduction or *PI3K* *p85* expression (claimed). Antisense compounds are commonly used as research reagents and diagnostics. Antisense compounds either alone or in combination with other antisense compounds or therapeutics...

... of powders or aerosols, including by nebulizer, intratracheal, intranasal, epidermal and transdermal, oral or parenteral. No specific dosage is given. EXAMPLE - Antisense compound that inhibits *PI3K* *p85* was synthesized by standard solid phase synthesis. (121 pages)

DESCRIPTORS: human recombinant *PI3K* *p85* protein prep., isol., antisense, appl. cancer, *diabetes*, obesity, inflammation diagnosis, prevention, therapy animal mammal tumor DNA sequence protein sequence (21, 51)

4/3,K/12 (Item 2 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

0253939 DBR Accession No.: 2000-08429

Restored *insulin*-sensitivity in IRS-1-deficient mice treated by adeno virus-mediated gene therapy - adeno virus vector-mediated *insulin* receptor substrate-1 gene transfer, used in *diabetes* gene therapy

AUTHOR: Ueki K; Yamauchi T; Tamemoto H; Tobe K; Yamamoto-Honda R; Kaburagi Y; Akanuma Y; Yazaki Y; Aizawa S; Nagai R; +Kadowaki T
CORPORATE AFFILIATE: Univ.Tokyo Asahi-Life-Found. Univ.Kumamoto
CORPORATE SOURCE: Department of Internal Medicine, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan. email:kadowaki-3im@h.u-tokyo.ac.jp

JOURNAL: J.Clin.Invest. (105, 10, 1437-45) 2000
ISSN: 0021-9738 CODEN: JCINAO
LANGUAGE: English

Restored *insulin*-sensitivity in IRS-1-deficient mice treated by adeno virus-mediated gene therapy - adeno virus vector-mediated *insulin* receptor substrate-1 gene transfer, used in *diabetes* gene therapy

ABSTRACT: *Insulin* resistance is a common symptom of both overt *diabetes* and susceptibility to developing *diabetes*. As a result maintaining or restoring *insulin* sensitivity may be a means of preventing disease. Homozygous disruption of *insulin* receptor substrate-1 (IRS-1) in mice has been shown to cause *insulin* resistance without disease development. The mechanism of systemic *insulin* resistance was explored, and adeno virus vector mediated gene transfer was used to restore *insulin* sensitivity. Mice transformed by the adeno virus encoding IRS-1 exhibited almost normal *insulin* sensitivity. When the transgene was modified by deletion of the phosphatidylinositol 3-kinase (*PI3K*) *p85* subunit binding site (IRS-1dp85), *insulin* sensitivity was also restored, even though PI3K is known to play a vital function in *insulin*'s metabolic responses. Protein kinase-B (PKB) activity in the liver was reduced in null mice relative to wild-type or null mice expressing IRS...

.. and PKB activity in primary hepatocytes of the null mice, but IRS-1dp85 only altered PKB activity, indicating PKB activation could be used to reduce *insulin* resistance. (40 ref)

DESCRIPTORS: adeno virus vector-mediated *insulin* receptor substrate-1 gene transfer, expression in hepatocyte, enhanced protein-kinase-B act., appl. *diabetes* prevention, gene therapy cloning liver (Vol.19, No.15)

4/3,K/13 (Item 1 from file: 35)

DIALOG(R)File 35:Dissertation Abs Online

01867809 ORDER NO: AADAA-I3038640

Behavioral, metabolic and molecular correlates of *insulin* sensitivity in humans

Author: Ho, Richard Casey

Degree: Ph.D.

Year: 2001

Corporate Source/Institution: Colorado State University (0053)

Source: VOLUME 63/01-B OF DISSERTATION ABSTRACTS INTERNATIONAL. PAGE 186. 124 PAGES

ISBN: 0-493-51484-8

Behavioral, metabolic and molecular correlates of *insulin* sensitivity in humans

Whole body *insulin* resistance appears to precede many of the metabolic abnormalities that are involved in the progression toward type 2 *diabetes* mellitus (T2DM), obesity, dyslipidemia, hypertension, cardiovascular disease and some cancers. The overall objective of this project was to characterize modifiable correlates and sequelae that are associated with *insulin* sensitivity in humans.

In <italic>Study 1< italic>, we determined whether differences in *insulin* sensitivity persist between nonobese, nondiabetic Mexican American (MA) (n = 13; 27.0 ± 2.0 yrs; BMI = 23.0 ± 0.7) and Non-Hispanic...

..plusmn; 1.5 yrs; BMI = 22.8 ± 0.6) males and females after accounting for effects of exercise, adiposity, dietary intake and skeletal muscle *insulin* signaling protein abundance. Significant differences in *insulin* sensitivity, estimated by the homeostatic model assessment of *insulin* resistance, between MA and NHW persisted (1.53 ± 0.22 vs. 0.87 ± 0.16, p < 0.05, respectively) after accounting for effects of acute and chronic exercise, and adiposity. Protein levels of IRβ, *PI3K* *p85*, Akt1, Akt2 and GLUT4 were not different between the two groups. Differences in HOMA-IR scores lost significance after accounting for percent intake of palmitic acid, palmitoleic acid and skeletal muscle protein abundance of IRβ. Our results suggest that differences in *insulin* sensitivity between nonobese, nondiabetic MA and NHW are not due to differences in level of cardiorespiratory fitness or adiposity, however dietary intake and key *insulin* signaling protein levels could contribute to these ethnic differences.

In <italic>Study 2< italic>, we determined whether the TNF-α system accounted for differences in *insulin* sensitivity between MA (n = 13; 27.0 ± 2.0 yrs, BMI = 23.0 ± 0.7) and NHW (n = 13; 24.8 ± 1.5 yrs; BMI = 22.8 ± 0.6) subjects. MA were less *insulin* sensitive compared to NHW, while circulating levels of TNF-α were higher (3.11 ± 0.38 vs. 2.10 ± 0.24 pg/ml...

...127.36 pg/ml, p < 0.05) were significantly lower among MA subjects. TNFα, sTNFR1 and sTNFR2 were not related to estimates of *insulin* sensitivity or abdominal fat patterning when the two groups were analyzed in aggregate. These data indicate that although circulating levels of TNFα and sTNFR2 are different between nonobese, nondiabetic MA and NHW, they do not account for the observed differences in *insulin*

sensitivity

In <italic>Study 3</italic>, we determined the relationship between various estimates of *insulin* sensitivity, LDL size and oxidized LDL in a group of overweight, nondiabetic males (N = 34, BMI 25–35 kg m<super>2</super>, 50–75y). Estimates of *insulin* sensitivity were inversely related to LDL size (r = .41, <italic>P</italic> < 0.05), although these relationships were largely mediated by VLDL triglyceride and HDL cholesterol concentrations. Estimates of *insulin* sensitivity were not related to plasma oxidized LDL concentrations. Furthermore, LDL size was not significantly associated with oxidized LDL. In this homogeneous group of overweight, nondiabetic men, estimates of *insulin* sensitivity are not independent markers of atherogenic small, dense and oxidized LDL.

4/3,K/14 (Item 2 from file: 35)

DIALOG(R)File 35:Dissertation Abs Online

01769574 ORDER NO: AADAA-I9988612

Roles of phosphoinositide 3-kinase (*PI3K*) *p85* regulatory subunits in development and physiology

Author: Yballe, Claudine Marie
Degree: Ph.D.
Year: 2000
Corporate Source/Institution: Harvard University (0084)
Source: VOLUME 61/09-B OF DISSERTATION ABSTRACTS INTERNATIONAL. PAGE 4521. 250 PAGES
ISBN: 0-599-96166-X

Roles of phosphoinositide 3-kinase (*PI3K*) *p85* regulatory subunits in development and physiology

...means of determining the physiological role of PI3K. Overall, these mice are viable and fertile. I examined B cell development and function as well as *glucose* homeostasis and compared these results to those reported in mice lacking p85α. Also discussed briefly is the analysis of mice lacking both p85&beta...

...similar as well as opposite defects to p85α null mice. This dichotomy is useful in trying to dissect the roles of each isoform physiologically.

Insulin and *glucose* metabolism in p85β null mice are also defective in both similar and opposite ways to that of p85α. Like p85α null mice they show increased *insulin* sensitivity, but contrary to what is reported in p85α<super>−/−</super> and p85α<super>+/− </super> mice which have improved *glucose* tolerance over wild-type, they show *glucose* intolerance.

Given that the p85 subunits have different as well as similar defects, we were curious to determine the phenotype of the double knockout. The...

4/3,K/15 (Item 1 from file: 149)

DIALOG(R)File 149:TGG Health&Wellness DB(SM)

02051695 SUPPLIER NUMBER: 82320720 (USE FORMAT 7 OR 9 FOR FULL TEXT)

Autocrine activation of the IGF-I signaling pathway in mesangial cells isolated from *diabetic* NOD mice.(*insulin*-like growth factor 1)

Tack, Ivan; Elliot, Sharon J.; Potier, Mylene; Rivera, Ana, Striker, Gary E.; Striker, Liliane J.
Diabetes, 51, 1, 182(7)
Jan,
2002
PUBLICATION FORMAT: Magazine/Journal; Refereed ISSN: 0012-1797
LANGUAGE: English RECORD TYPE: Fulltext TARGET AUDIENCE: Professional
WORD COUNT: 4662 LINE COUNT: 00368

Autocrine activation of the IGF-I signaling pathway in mesangial cells isolated from *diabetic* NOD mice.(*insulin*-like growth factor 1)

TEXT:

Mesangial cells isolated from NOD mice after the onset of *diabetes* have undergone a stable phenotypic change. This phenotype is characterized by increased expression of IGF-I and downregulation of collagen degradation, which is associated with...
...2 activity. Here, we investigated the IGF-I signaling pathway in mesangial cells isolated from NOD mice before (nondiabetic NOD mice (ND-NOD)) and after (*diabetic* NOD mice (D-NOD)) the onset of *diabetes*. We found that the IGF-I signaling pathway in D-NOD cells was activated by autocrine IGF-I. They had phosphorylation of the IGF-I receptor (beta)-subunit, phosphorylation of *insulin* receptor substrate (IRS)-I, and association of the p85 subunit (phosphatidylinositol 3-kinase (PI3K)) with the IGF-I receptor and IRS-1 in D-NOD...

...change in D-NOD cells is associated with constitutive activation of the IGF-I signaling pathways, which may participate in the development and progression of *diabetic* glomerulosclerosis. *Diabetes* 51: 182-188, 2002

Alterations in the availability of IGF-I or an altered response to IGF-I may play a role in *diabetic* nephropathy. Mesangial cells are critical determinants in the accumulation of extracellular matrix (ECM) in the glomeruli of patients with *diabetic* nephropathy (1). These cells express IGF-I receptors and synthesize IGF-I (2-6). We have shown that glomerular mesangial cells isolated from mice with autoimmune type 1 *diabetes* (NOD) exhibited a stable phenotypic change after the onset of *diabetes* (7). This stable change was characterized by increased IGF-I synthesis and increased cell proliferation. After blocking autocrine IGF-I with a neutralizing antibody, the number of detectable IGF-I surface receptors was found to be increased approximately threefold in *diabetic* NOD mice (D-NOD) compared with that in cells isolated from nondiabetic NOD mice (ND-NOD). This stable phenotypic change may be present in other experimental models of *diabetic* nephropathy because comparable phenotype changes were found in mesangial cells isolated from a model of spontaneously occurring type 2 *diabetes* with nephropathy (db/ db) after the onset of *diabetes* (8). We showed that excess IGF-I secretion by mesangial cells could contribute to extracellular matrix deposition in *diabetic* nephropathy through a decrease in MMP-2 synthesis (9). A preliminary report in which mesangial cells from patients with type 2 *diabetes* and nephropathy had an altered phenotype (10) suggested that this observation may apply to patients.

We compared intracellular IGF-I signaling pathways in mesangial cells isolated from NOD mice before and after the spontaneous onset of *diabetes* to determine whether the phenotypic change in mesangial cells isolated from *diabetic* mice resulted from changes in these pathways. The IGF-I signaling pathway was intact in mesangial cells isolated from both D-NOD and ND-NOD...

...PAGE and immunoblotting were obtained from Novex (San Diego, CA). HEPES, phenylmethylsulfonyl fluoride (PMSF), aprotinin, leupeptin, benzamidine, EDTA, sodium pyrophosphate, sodium fluoride and sodium orthovanadate, *insulin*, Triton X-100, Tween 20, bovine serum albumin (BSA; Fraction V), glycerol, and NaCl were from Sigma Chemical (St. Louis, MO). PD98059 and LY294002 were...

...Cruz (Santa Cruz, CA).

Isolation and propagation of mesangial cell lines. Mesangial cells were isolated from NOD mice before and after the spontaneous onset of *diabetes* as previously described (7) Briefly, glomeruli were isolated from kidneys of 4- to 6-month-old D-NOD and ND-NOD mice. *Diabetic* mice had glycosuria for 4-8 weeks before sacrifice and were receiving two *insulin* injections each day. Nondiabetic mice had normal *glucose* tolerance, as determined by a *glucose* tolerance test before sacrifice. Several lines of mesangial cells were derived from each of several D-NOD and ND-NOD mice (7). In each experiment...

...were plated in either 6-well plates or T75 (cm sub.2) flasks in B medium containing 20% fetal bovine serum and 6 mmol/l *glucose* as previously described (7). Twenty-four hours before collection, the medium was replaced with B medium containing 0.1% BSA. Cell number was determined in duplicate wells at each experimental time point. Phosphorylation of IGF-I receptor and *insulin* receptor substrate (IRS)-1 and IRS-2 were examined after exposure to either IGF-I (50 ng/ml) or *insulin* (50 ng/ml) for 10 min. To block activation of the signaling pathway, a neutralizing IGF-I antibody (34 (microg/ml), PD98059 or a...

...20,000g for 30 min at 4 (degrees) C. Samples were analyzed by electrophoresis through 6% (IRS-1, IRS-2, and IGF-IR(beta)), 8% (*PI3K*-*p85*), and 10% (ERK-1/2 and phospho-ERKs) polyacrylamide gels and electrophoretically transferred to nitrocellulose membranes. After overnight incubation at 4 (degrees) C in Tris...

...1% milk plus 1% BSA and 0.05% Tween-20, the blots were exposed to antibodies recognizing either IRS-1, IRS-2, IGF-IR(beta), *PI3K*-*p85*, ERK-1.2, phospho-ERKs, and antiphosphotyrosine PY20 or PY99 for 1 h at room temperature. The primary antibodies were revealed using the corresponding goat...was boiled for 3 min before analysis as described above.

Zymography for matrix metalloproteinases. To determine whether blocking IGF-I activation increased MMP-2 in *diabetic* mesangial cells, medium was collected from cells exposed to PD98059 for 24 h or LY294002 for 36 h. Cell supernatants were electrophoresed and incubated for...

...50 ng/ml IGF-I resulted in a prominent phosphorylation of the IGF-I receptor (beta)-chain. Interestingly, whereas incubation with a similar dose of *insulin* had no effect on phosphorylation of the (beta)-chain of the IGF-I receptor, it induced phosphorylation of a band migrating above the IGF-I receptor (Fig. 3, middle panel). We postulated that the protein phosphorylated by *insulin* may be the (beta)-chain of *insulin* receptor, based on its homology with the (beta)-chain of IGF-I receptor and its molecular weight (14).

(FIGURE 3 OMITTED)

The p85 subunit of...

...associate with the (beta)-chain of the IGF-I receptor. The amount of p85 associated with IGF-I receptor was greater in mesangial cells from *diabetic* mice (Fig. 3, lower panel). The addition of IGF-I to media increased the association of the p85 subunit to the IGF-I receptor in...

...1 and -2 were detected. Examination of mesangial cells in the basal state revealed that phospho-IRS-1 was increased in those cells isolated from *diabetic* mice compared with those isolated from ND-NOD mice. A 10-min exposure to 50 ng/ml IGF-I resulted in a twofold increase in IRS-1 phosphorylation, whereas IRS-2 phosphorylation was unchanged. Stimulation with 50 ng/ml *insulin* had no effect, consistent with the fact that there are few *insulin* receptors on the surface of glomerular mesangial cells (2,3).

(FIGURE 4 OMITTED)

As has been reported, there was also spontaneous association of the p85...

...are activated by phosphorylation of their tyrosine residues (16). ERK1 and -2 were expressed at similar levels in all mesangial cell lines, irrespective of the *diabetic* status of the NOD mice (Fig. 5A). The phosphorylated (activated) forms of ERK1 and -2 in the basal state were examined by Western immunoblotting using...increased in mesangial cells isolated from D-NOD mice when compared with those from nondiabetic littermates. These data are consistent with our previous report that *diabetic* cells had more surface IGF-I receptors when autocrine IGF-I was blocked with a neutralizing antibody to IGF-I (7). In addition, the increase...

...manner. Similarly, there was increased phosphorylation of tyrosine residues and an increased association of the P85 subunit to the receptor in

mesangial cells isolated from *diabetic* mice. In addition, whereas ERK1 and -2 levels were found to be similar in all cell lines from *diabetic* and nondiabetic mice, activated (phosphorylated) ERK2 was increased only in the basal state of cells isolated from *diabetic* mice. Although the addition of exogenous IGF-I further increased levels of phosphorylated ERKs in D-NOD cells, the increase was less prominent than in...
...et al. (9) reported the induction of tyrosyl phosphorylation of nuclear proteins by IGF-I in mesangial cells isolated from a model of type 2 *diabetes* (db/db). However, to our knowledge, there are no reports on the activation of this pathway in mesangial cells isolated from a model of type 1 *diabetes*, such as the NOD mouse.

Because cells isolated from D-NOD mice synthesized more IGF-I at baseline (7), we added a neutralizing antibody to...

...IGF receptor (beta)-subunit, reduced the amount of phospho-IRS-1, and decreased the amounts of phosphorylated ERK1 and -2 in the cells isolated from *diabetic* mice.

Finally, because the amount of p85 subunit associated with the IGF receptor was increased in cells isolated from *diabetic* mice, we used an inhibitor (LY294002) to determine whether downstream signaling events were affected. Importantly, after the addition of LY294002, we found decreased levels of...data suggest that overexpression of IGF-I may result in decreased degradation of ECM and lead to an accumulation of ECM, a characteristic feature of *diabetic* nephropathy. A recent report suggests that constitutive activation of MEK (MEK1, upstream from ERK) leads to activation of MMP-2 in a rat fibroblast cell line (11). In contrast, we found that activation of ERK1 and -2 is associated with decreased MMP-2 activity in *diabetic* mesangial cells, since blocking IGF-I activation through either the PI3K or MAPK pathway increased MMP-2 activity. This suggests that MAPK regulation of MMP...

...components (ERK1 and -2). This may lead to decreased ECM degradation and appears to be part of the phenotypic changes induced after the onset of *diabetes*.

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...contributed equally to this work.

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BSA, bovine serum albumin; D-NOD, *diabetic* NOD mice; ECM, extracellular matrix; ERK, extracellular response kinase; IRS, *insulin* receptor substrate; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/extracellular response kinase kinase; ND-NOD, nondiabetic NOD mice; PI3K, phosphatidylinositol 3-kinase...

DESCRIPTORS: *Insulin*-like growth factor 1...

...*Diabetic* nephropathies

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Regulation by *insulin* of gene expression in human skeletal muscle and adipose tissue: evidence for specific defects in type 2 *diabetes*.
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Regulation by *insulin* of gene expression in human skeletal muscle and adipose tissue: evidence for specific defects in type 2 *diabetes*.

TEXT:

Defective regulation of gene expression may be involved in the pathogenesis of type 2 *diabetes*. We have characterized the concerted regulation by *insulin* (3-h hyperinsulinemic clamp) of the expression of 10 genes related to *insulin* action in skeletal muscle and in subcutaneous adipose tissue, and we have verified whether a defective regulation of some of them could be specifically encountered in tissues of type 2 *diabetic* patients. Basal mRNA levels (determined by reverse transcriptase-competitive polymerase chain reaction) of *insulin* receptor, *insulin* receptor substrate-1, p85(alpha) phosphatidylinositol 3-kinase (PI3K), p110(alpha)PI3K, p110(beta)PI3K, GLUT4, glycogen synthase, and sterol regulatory-element-binding protein-1c (SREBP-1c) were similar in muscle of control (n = 17), type 2 *diabetic* (n = 9), type 1 *diabetic* (n = 9), and nondiabetic obese (n = 9) subjects. In muscle, the expression of hexokinase II was decreased in type 2 *diabetic* patients (P < 0.01). In adipose tissue, SREBP-1c (P < 0.01) mRNA expression was reduced in obese (nondiabetic and type 2 *diabetic*) subjects and was negatively correlated with the BMI of the subjects (r = -0.63, P = 0.02). *Insulin* ((+ or -) 1,000 pmol/l) induced a two- to threefold increase (P < 0.05) in hexokinase II, p85(alpha)PI3K, and SREBP-1c mRNA levels in muscle and in adipose tissue in control subjects, in *insulin*-resistant nondiabetic obese patients, and in hyperglycemic type 1 *diabetic* subjects. Upregulation of these genes was completely blunted in type 2 *diabetic* patients. This study thus provides evidence for a specific defect in the regulation of a group of important genes in response to *insulin* in peripheral tissues of type 2 *diabetic* patients. *Diabetes* 50: 1134-1142, 2001

Insulin resistance is the main metabolic feature of type 2 *diabetes* (1,2), and several studies indicate that it generally precedes the onset of the disease (2,3). In vivo, skeletal muscle is the major site for *insulin*-dependent *glucose* disposal, and type 2 *diabetic* patients are characterized by a marked decrease in *insulin*-stimulated *glucose* utilization in muscle mainly due to reduced *glucose* uptake and storage (1,2). *Insulin* stimulates *glucose* uptake by increasing the translocation of GLUT4-containing vesicles to the plasma membrane and by modifying the activity of enzymes involved in *glucose* metabolism (4). *Insulin* action is initiated by binding of the hormone to cell membranes and activation of the *insulin* receptor tyrosine kinase that results in the stimulation of intracellular signaling cascades (4). Among these cascades, the phosphatidylinositol 3-kinase (PI3K) pathway is thought to play a crucial role in the effects of *insulin* on *glucose* metabolism (5). Several defects in the *insulin* signaling pathways have been identified in skeletal muscle of type 2 *diabetic* patients. Impaired phosphorylation of *insulin* receptor and *insulin* receptor substrate (IRS)-1 in response to *insulin* has been reported (6-8), and the induction of PI3K and Akt kinase activities have been found to be reduced (8-10). The stimulation of glycogen synthase activity is also decreased in skeletal muscle of patients with type 2 *diabetes* (11). All of these alterations take place at the level of the acute posttranslational regulation of key enzyme activity.

In addition to this level of regulation, *insulin* also controls the transcription of important genes in its target cells (12). This action is crucial for *insulin* to sustain its metabolic effects and also to adapt the organism to environmental changes. It is well known that environmental factors play a major role in type 2 *diabetes*, in addition to genetic predisposition. Alterations in the transcriptional mechanisms involved in the adaptation of the cells to environmental changes may thus participate in the pathogenesis of the disease (13,14). In keeping with this hypothesis, the expression of some important genes involved in *insulin* action and *glucose* metabolism has been found to be altered in peripheral tissues of type 2 *diabetic* patients. For example, the basal expression levels of hexokinase II (15,16) and glycogen synthase (17) in skeletal muscle and of GLUT4 (18) and IRS...

...reported that the induction of GLUT4 (20,21) and hexokinase II (22) expression in response to hyperinsulinemia is impaired in skeletal muscle of type 2 *diabetic* patients. Recently, we have demonstrated that the regulation by *insulin* of the p85(alpha) regulatory subunit of *PI3K* (*p85*(alpha)PI3K) is also altered in muscle and adipose tissue of patients with type 2 *diabetes* (23). Taken together, these data suggest that the regulation of a cluster of genes involved in *insulin* action and *glucose* metabolism may be affected in type 2 *diabetes*. It could thus be hypothesized that a common mechanism, involved in the regulation of these genes and probably others that have yet to be identified, is altered in the peripheral tissues of type 2 *diabetic* patients.

However, this hypothesis is based on results from unrelated studies that have been performed using different hyperinsulinemic conditions and different procedures to estimate gene expression levels. Moreover, in most of the studies, the regulation of one individual gene was investigated, although we have reported the regulation of three mRNAs (*insulin* receptor, IRS-1, and p85(alpha)PI3K) in parallel (23). It is thus difficult to conclude whether the same mechanism is involved in the defective...

...In addition, it is still unclear whether the observed defects in the regulation of gene expression result from a specific alteration linked to type 2 *diabetes* or are secondary to the metabolic state of the patients, such as *insulin* resistance or chronic hyperglycemia.

The present study was performed with the aim of verifying these points. To this end, we investigated the concerted regulation by *insulin* of the expression of 10 candidate genes, measured by similar reverse transcriptase--competitive polymerase chain reaction (RT-cPCR) assays, in skeletal muscle and in adipose tissue of type 2 *diabetic* patients. In addition to the regulation of genes that have already been studied individually (20-24), we also investigated, for the first time, the regulation...

...To verify whether an impaired regulation of the expression of some of these genes is specific or secondary to the metabolic state of type 2 *diabetes*, age-matched control subjects, type 2 *diabetic* patients, and *insulin*-resistant nondiabetic obese subjects were investigated in parallel. Moreover, the contribution of a deleterious role of chronic hyperglycemia was verified with a group of type 1 *diabetic* subjects with Hb(A.sub.1c) similar to that in the type 2 *diabetic* patients.

RESEARCH DESIGN AND METHODS

Subjects. The characteristics of the 44 subjects involved in the study are presented in Table 1. The 17 healthy lean volunteers were divided into two groups on the basis of their age. None of these subjects had impaired *glucose* tolerance or a familial or personal history of *diabetes*, obesity, dyslipidemia, or hypertension. One group of control subjects (six women mid three men, age 51 (+ or -) 2 years, BMI 23 (+ or -) 1 kg/(m.sup.2)) was age-matched with nine patients with type 2 *diabetes* (three women and six men, age 54 (+ or -) 3 years, BMI 30 (+ or -) 1 kg/(m.sup.2), duration of *diabetes* 7 (+ or -) 1 years) and nine nondiabetic obese subjects (six women and three men, age 43 (+ or -) 4 years, BMI 35 (+ or -) 1 kg/(m.sup.2)). The type 2 *diabetic* patients interrupted, under medical control, their usual treatment with oral antidiabetic agents at least 1 week before the investigation. None of the obese subjects had impaired *glucose* tolerance as assessed by classic oral *glucose* tolerance test. The metabolic data of some of these subjects (all of the control and type 2 *diabetic* subjects and six of the nine obese patients) have been presented in a previous study (23). An unrelated group of healthy subjects (seven women and one man, age 25 (+ or -) 1 years, BMI 22 (+ or -) 1 kg/(m.sup.2)) served as control subjects for a group of type 1 *diabetic* patients (five women and four men, age 33 (+or -) 3 years, BMI 23 (+ or -) 1 kg/(m.sup.2), duration of *diabetes* 16 (+ or -) 3 years, C-peptide <0.05 ng/ml) with Hb(A.sub.1c) (9.2 (+ or -) 0.3%) similar to that in the type 2 *diabetic* subjects (10.9 (+ or -) 0.3%, P = 0.252). Type 1 *diabetic* patients had no familial antecedent of type 2 *diabetes*, none had complications, and they were all treated with daily injections of *insulin* (45 (+ or -) 5 IU/day). The last dose of *insulin* was administered the day before beginning the clamp study. On the morning of

the experiment, type 1 *diabetic* patients showed marked hyperglycemia (12.6 (+ or -) 0.8 mmol/l) that, was not significantly different from the glycemia of the type 2 *diabetic* subjects (10.5 (+ or -) 0.6 mmol/l, P = 0.401). To avoid further increase in plasma *glucose* concentrations during the 3 h of the basal period that preceded the hyperinsulinemic clamp, an intravenous low-dose *insulin* infusion (1.5 IU/h) was administrated to the type 1 *diabetic* subjects. This infusion was maintained during the 3 h of the basal period so that both type 1 and type 2 *diabetic* patients had similar levels of hyperglycemia at the beginning of the *insulin* clamp period.

All participants gave their written consent after being informed of the nature, purpose, and possible risks of the study. The experimental protocol was...

.. Hospices Civils de Lyon and performed according to the French legislation (Huriet law).

Study design All studies were performed after an overnight fast. So that *insulin* action on *glucose* metabolism and on target gene expression could be investigated, the subjects were submitted to a 3-h euglycemic-hyperinsulinemic clamp (24).

Euglycemic-hyperinsulinemic clamp. Before the hyperinsulinemic period, basal *glucose* turnover rate was determined during the last 30 min of a 3-h basal period by tracer dilution methodology using a primed (6,6-(sup.2)H2)*glucose* (Eurisotop, St. Aubain, France) infusion (0.02 mg * (kg.sup.-1) * (min.sup.-1)). Then, a 3-h euglycemic-hyperinsulinemic clamp was started by the infusion of *insulin* (Actrapid Novo, Copenhagen, Denmark) at a rate of 450 pmol * (m.sup.-2) * (min.sup.-1). Primed (6,6-(sup.2)H2)*glucose* was infused (0.1 mg * (kg.sup.-1) * (min.sup.-1)) during the clamp to determine *glucose* turnover rate, while any decrease in blood *glucose* was prevented by adapted infusion of 20% *glucose* solution (Aguettant, Lyon, France). For the determination of metabolites, hormones, and (6,6-(sup.2)H2)*glucose* isotopic enrichment, blood samples were drawn every 10 min during the last 30 min of the basal and hyperinsulinemic periods Metabolite and hormone concentrations were measured using enzymatic methods and radioimmunoassays. Plasma isotopic enrichment of (6,6-(sup.2)H2)*glucose* was determined by gas chromatography-mass spectrometry (5,971 MSD; Hewlett-Packard, Palo Alto, CA), and *glucose* turnover rates were calculated using steady-state equations as previously described (25). For the diabolic patients, glucosuria was subtracted from *glucose* turnover rates to calculate *glucose* utilization. For the young control and the type 1 *diabetic* subjects, (6,6-(sup.2)H2)*glucose* was not used and thus basal *glucose* disposal was not determined. For these subjects, the rates of *glucose* infusion during the clamp were used to estimate whole-body *glucose* disposal rates, since it has already been established that endogenous *glucose* production is suppressed with the level of hyperinsulinemia reached during the clamp (26). To estimate *glucose* and lipid oxidation rates, respiratory exchange measurements were performed during the final 30 min of both the basal and the hyperinsulinemic periods, using a flow...

...the vastus lateralis muscle using Weil-Blakesley pliers. The size of the biopsies averaged 60 mg, with no difference between samples from control, obese, and *diabetic* subjects or before and after clamp. Abdominal subcutaneous adipose tissue was aspirated from the periumbilical area through a 15-gauge needle (27). About 250 mg...

...1.2 (+ or -) 0.1 (micro)g/100 mg of adipose tissue (wet weight), and were not significantly different in tissues from control, obese, and *diabetic* subjects, before or after the clamp. Total RNA solutions were stored at -80 (degrees) C until quantification of the target mRNAs.

Quantification of messenger RNAs...using a large range of in vitro synthesized RNA (0.25-50 amol in the reaction) as recommended (29).

To accurately determine the effect of *insulin*, total RNA of the two muscle (or adipose tissue) biopsies from the same individual (before and after clamp) were prepared simultaneously and the assays of...

...analyzed using Spearman's rank correlation test. The threshold for

significance was set at P < 0.05.

RESULTS

Characteristics of the subjects and effects of *insulin* on *glucose* metabolism (Table 1). After the subjects fasted overnight, plasma concentrations of *insulin*, nonesterified fatty acids, and triglycerides were higher in obese and type 2 *diabetic* patients than in lean control subjects. Type 1 and type 2 *diabetic* patients had higher fasting glycemia. Basal *glucose* disposal rate was slightly reduced in obese patients compared with age-matched control subjects and tended to be higher in type 2 *diabetic* patients, although the difference was not significant (P = 0.09). During the hyperinsulinemic clamp, the stimulation by *insulin* of *glucose* utilization rate was profoundly reduced by >50% in obese and in type 2 *diabetic* patients. Both *insulin*-stimulated nonoxidative *glucose* disposal and *glucose* oxidation rates were decreased when compared with healthy lean subjects. In addition, nonesterified fatty acid concentrations remained higher in obese (56 (+ or -) 5 (micro)mol/l, P = 0.003) and type 2 *diabetic* patients (74 (+or -) 10 (micro)mol/l, P = 0.002) than in age-matched control subjects (29 (+ or -) 5 (micro)mol/l) during the clamp. Type 1 *diabetic* patients displayed a slight reduction (17%) in *glucose* disposal rate during the clamp, which was very close to being significant (P = 0.053) (Table 1). This result thus indicates that the type 1 *diabetic* patients involved in this study were not characterized by a marked *insulin* resistance, in contrast to the obese and type 2 *diabetic* subjects.

Basal mRNA expression pattern in skeletal muscle. We have first investigated the regulation of the expression of nine genes that encode major proteins and enzymes related to *insulin* action on *glucose* metabolism. They include *insulin* receptor, IRS-1, p85(alpha)PI3K, p110(alpha)PI3K, p110(beta)PI3K, Ras protein associated with *diabetes* (Rad), GLUT4 hexokinase II, and glycogen synthase. The basal concentrations of the nine transcripts in vastus lateralis muscle, determined by RT-cPCR, are presented in...

...mRNA. When the data from the five groups were compared (Kruskal-Wallis analysis), there was no statistical difference between groups in the mRNA levels of *insulin* receptor, IRS-1, p85(alpha)PI3K, p110(...0.001) mRNA levels. When the same analysis was performed taking into account the data of the age-matched control, nondiabetic obese, and type 2 *diabetic* subjects, the differences remained significant for hexokinase II (P < 0.001) only. Using the nonparametric Mann-Whitney's test, we found that the mRNA levels of hexokinase II were significantly reduced in the skeletal muscle of type 2 *diabetic* patients with respect to both the control (P = 0.007) and the nondiabetic obese (P = 0.003) subjects. Regarding Rad, Fig. 1 shows that there was a twofold reduction in Rad mRNA levels in the groups of young subjects (control and type 1 *diabetic* subjects) when compared with the groups of older subjects (P < 0.001), suggesting an age-related difference in the expression of this gene. When all...

...P = 0.008) between Rad mRNA and the age of the subjects. Importantly, there was no difference in the expression of Rad between type 2 *diabetic* patients and age-matched healthy control subjects (P = 0.954) or age-matched nondiabetic obese subjects (P = 0.391). Glycogen synthase mRNA levels tended to be reduced in the muscle of type 2 *diabetic* patients when compared with their age-matched control subjects, and the difference was very close to being significant (P = 0.064). On the other hand, type 1 *diabetic* patients showed a tendency for an increased expression of p85(alpha)PI3K and GLUT4 mRNAs, but the difference was not significant when tested using the Kruskal-Wallis analysis (P = 0.222 and 0.144 for GLUT4 and p85(alpha)PI3K mRNA, respectively). However, when the type 1 *diabetic* patients were directly compared with the young control subjects (Mann-Whitney's test), the *diabetic* patients had a slightly higher GLUT4 mRNA levels (64 (+ or -) 9 vs. 44 (+ or -) 3 amol/(micro)g total RNA, P = 0.043).

[FIGURE 1 OMITTED]

Effects of 3-h *insulin* infusion on mRNA expression. Table 2 shows the *insulin*-induced changes in mRNA levels of the nine target genes in the skeletal muscle of the five groups of subjects. The data are presented

as...

...preclamp) values. In the two groups of healthy control subjects, the mRNA levels of p85(alpha)PI3K, hexokinase II, and Rad were markedly increased by *insulin*. The effect on GLUT4 expression was less pronounced (1.5-fold increase), but it was highly significant (P = 0.011). In contrast to these four genes, the expression of IRS-1 was significantly reduced after the hyperinsulinemic clamp. Finally, the mRNA levels of *insulin* receptor, the p110(alpha) and p110(beta) catalytic subunits of PI3K, and glycogen synthase were not significantly modified by *insulin* infusion in control subjects. The effects of *insulin* in type 1 *diabetic* patients were similar to what was observed in the control subjects, with significant increases in the mRNA levels of p85(alpha)PI3K, hexokinase II, GLUT4, and Rad and a decrease in the expression of IRS-1 (Table 2)

In contrast, in *insulin*-resistant patients we identified several defects in the regulation of gene expression by *insulin*. The *insulin* -induced rise in p85(alpha)PI3K, hexokinase II, and GLUT4 mRNA levels was completely blunted in type 2 *diabetic* patients. Rad mRNA levels were still significantly increased by *insulin* in type 2 *diabetic* patients, but the effect of *insulin* appeared attenuated (~61% increase in *diabetic* vs. 200% increase in control subjects, P = 0.064). As in control subjects, *insulin* receptor and glycogen synthase mRNA expression did not change in type 2 *diabetic* subjects. In addition, *insulin* induced a significant decrease in p110(alpha) and p110(beta) PI3K mRNA concentrations in type 2 *diabetic* muscle that was not observed in any of the other groups (Table 2). Finally, in *insulin*-resistant nondiabetic obese subjects, an impaired regulation by *insulin* was observed only for GLUT4 mRNA. All other transcripts responded to *insulin* in the same way and with similar magnitude in nondiabetic obese and in healthy lean subjects (Table 2).

Figure 2 shows the individual data regarding the regulation by *insulin* of p85(alpha)PI3K and hexokinase II mRNA levels in skeletal muscle, clearly demonstrating that there was a specific defect in the skeletal muscle of type 2 *diabetic* subjects that was not encountered in *insulin*-resistant obese subjects and in hyperglycemic type 1 *diabetic* patients.

(FIGURE 2 OMITTED)

Regulation of gene expression in adipose tissue. We further investigated whether the impaired regulation of gene expression observed in skeletal muscle also existed in adipose tissue of type 2 *diabetic* patients. Due to low yield in total RNA recovery in adipose tissue (~1 (micro)g/100 mg tissue), we did not measure the mRNA levels of all target genes and we first studied *insulin* receptor, p85(alpha)PI3K, hexokinase II, and GLUT4 mRNA expression. *Insulin* receptor, p85(alpha)PI3K, and particularly hexokinase II mRNAs were expressed at higher levels in abdominal subcutaneous fat tissue than in skeletal muscle (Fig. 3). There was no significant difference regarding the mRNA levels of these three genes between the investigated groups (age-matched control, obese, and type 2 *diabetic* subjects). In contrast, basal GLUT4 mRNA expression was significantly reduced in adipose tissue in obese (17.4 (+ or -) 2.7 amol/(micro)g total RNA, P = 0.006) and in type 2 *diabetic* (13.3 (+ or -) 1.5 amol/(micro)g total RNA, P = 0.001) patients when compared with control subjects (46 (+ or -) 7.6 amol/(micro...

...was a significant negative correlation between GLUT4 mRNA levels and the BMI of the subjects (r = -0.68, P = 0.041). As in skeletal muscle, *insulin* markedly increased p85(alpha)PI3K (79 (+ or -) 27%, P = 0.018), hexokinase II (130 (+ or -) 27%, P = 0.018), and GLUT4 (114 (+ or -) 29%, P = 0.018) mRNA expression in adipose tissue of control subjects (Fig. 3). Similar positive effects of *insulin* were observed in nondiabetic obese patients (44 (+ or -) 15, 102 (+ or -) 32, and 64 (+ or -) 19% for p85(alpha)PI3K, hexokinase II, and GLUT4, respectively; P = 0.018). In contrast, Fig. 3 clearly shows that the effect of *insulin* on the mRNA expression of these three genes was, as in muscle, completely impaired in the adipose tissue of type 2 *diabetic* patients (18 (+ or -) 25%, P = 0.575, 1 (+ or -) 15%, P = 0.888 and 4 (+ or -) 11%, P = 0.401 for p85(alpha)PI3K, hexokinase...

... of SREBP-1c in skeletal muscle and adipose tissue. Recent evidence supports a crucial role of the transcription factor SREBP-1c in the effect of *insulin* on the transcription of several genes that encode enzymes of *glucose* and lipid metabolism (30-32). Therefore, SREBP-1c could potentially be involved in the impaired regulation of gene expression observed in tissues of type 2 *diabetic* patients. We set up a new RT-cPCR assay for SREBP-1c mRNA and studied its expression and regulation by *insulin* in skeletal muscle and adipose tissue from age-matched control, nondiabetic obese, and type 2 *diabetic* subjects (n = 8 per group). Figure 4 shows that SREBP-1c mRNA was more abundant in adipose tissue that in skeletal muscle in humans. In adipose tissue, the mRNA expression of SREBP-1c was significantly reduced both in the nondiabetic obese subjects (P = 0.005) and the type 2 *diabetic* patients (P = 0.009). Moreover, a significant negative correlation was found between SREBP-1c mRNA levels in adipose tissue and the BMI of the subjects ...

... 022). In skeletal muscle, the expression of SREBP-1c mRNA was not significantly different between groups, although it tended to be lower in type 2 *diabetic* patients than in control subjects (P = 0.082). Three hours of hyperinsulinemia produced a two- to threefold increase in SREBP-1c mRNA expression in skeletal. .

...was observed in tissues front nondiabetic obese subjects (P = 0.028 in muscle and P = 0.042 in adipose tissue). In contrast, the effect of *insulin* was completely impaired in tissues of type 2 *diabetic* patients (P = 0.124 in muscle and P = 0.123 in adipose tissue for the difference in SREBP-1c mRNA level after versus before clamp...
...other than those encoding GLUT4, hexokinase II, and p85(alpha)PI3K (20-23) could be altered, in a concerted manner, in tissues of type 2 *diabetic* patients. This was important to strengthen the hypothesis that, under the same experimental conditions, the regulation of a cluster of genes may be impaired during type 2 *diabetes*. To this end, we measured, in parallel in the same samples, the regulation by *insulin* of the expression of 10 candidate genes using validated RT-cPCR assays.

The second objective of the work was to define whether the observed defects in the regulation of gene expression resulted from a specific alteration in type 2 *diabetes* or were secondary to the metabolic state of the patients. Therefore, the regulation of the candidate genes was studied in tissues of healthy control subjects, type 2 *diabetic* patients, nondiabetic obese subjects, and type 1 *diabetic* patients, in parallel. These groups of subjects were selected to verify the contribution of either obesity-related *insulin* resistance (nondiabetic obese subjects) or chronic hyperglycemia (type 1 *diabetic* patients) on the defective regulation of gene expression observed in type 2 *diabetic* patients

The expression of GLUT4, hexokinase II, glycogen synthase, *insulin* receptor, and IRS-1 mRNAs in muscle or adipose tissue of type 2 *diabetic* patients has been previously reported (15-23). The between-group differences found in the present study were globally in agreement with what was previously observed for these genes (15-23). For example, we confirmed the marked reduction in the mRNA level of hexokinase II in the muscle of type 2 *diabetic* patients (15,22). Interestingly, we showed that this reduction was not observed in subcutaneous adipose tissue, indicating thus a tissue-specific alteration. In addition, our...

...We demonstrated that the basal mRNA expression of the p110(alpha) and p110(beta) catalytic subunits of PI3K was not altered in the muscle of *insulin*-resistant subjects. There is thus no defect in the basal expression of the main actors of *insulin* signaling (*insulin* receptor, IRS-1, and the regulatory and catalytic subunits of PI3K) in the skeletal muscle of type 2 *diabetic* patients. We also found that Rad mRNA concentration was similar in muscle of age-matched lean, obese, and type 2 *diabetic* subjects, confirming previous studies (33). Nevertheless, we observed a significant positive correlation between Rad mRNA and the age of the subjects, thus suggesting that Rad expression increases with age in skeletal muscle, independently of obesity and *diabetes*. Finally, we demonstrated that SREBP-1c mRNA expression is profoundly decreased in

subcutaneous adipose tissue of nondiabetic and type 2 *diabetic* obese subjects, while there was no significant difference between groups in skeletal muscle. The reduction in SREBP-1c mRNA in adipose tissue correlated with the...

...mice, using DNA microarray technology, that showed that the expression of SREBP-1c is two- to threefold decreased in white adipose tissue of obese and *diabetic* animals (34,35).

Hyperinsulinemia produced several changes in the mRNA levels of the investigated genes. Under our experimental conditions (*insulin* concentration of ~1,000 pmol/l for 3 h), these genes could be classified into the following four categories: 1) those that were not regulated by *insulin* in any of the groups of subjects; 2) those that are regulated (upregulated or downregulated) by *insulin* in a similar way in all groups; 3) those with an impaired regulation by *insulin* in *insulin* resistance (nondiabetic obese and type 2 *diabetic* patients); and 4) those with an altered regulation by *insulin* in type 2 *diabetic* patients specifically.

The mRNA expression of *insulin* receptor and glycogen synthase was not modified in skeletal muscle after 3 h of *insulin* infusion, in any of the groups. This was in agreement with most of the preceding studies (23,24,36).

IRS-1 mRNA levels were significantly reduced and Rad expression markedly increased in skeletal muscle in all groups, although the magnitude of the *insulin* effect on Rad mRNA seemed lower in type 2 *diabetic* patients. These results indicated thus that the regulation of IRS-1 and Rad mRNA expression are not significantly affected by obesity, *insulin* resistance, or *diabetes* in skeletal muscle.

The regulation by *insulin* of GLUT4 mRNA expression in muscle was altered both in nondiabetic obese and type 2 *diabetic* patients--the two groups of frankly *insulin*-resistant subjects. These data thus suggest that the impaired regulation of GLUT4 expression by *insulin* may result from the reduced *insulin* sensitivity of these patients. Interestingly, we found a normal induction of GLUT4 mRNA in response to *insulin* infusion in skeletal muscle of type 1 *diabetic* patients, whereas other investigators have previously reported an impaired regulation of GLUT4 expression in these subjects (26). Type 1 *diabetic* patients are classically considered as mildly *insulin* resistant (26,37,38). The difference between our results and those of Yki-Jarvinen et al. (26) are likely to be due to the higher levels of hyperinsulinemia maintained during the clamp in our study ((+ or -) 1,000 vs. 700 pmol/l). When moderate concentrations of *insulin* were used during the clamp, a significant reduction in *insulin*-induced *glucose* disposal rate has been observed in type 1 *diabetic* patients (26,37,38). Here, with a higher level of hyperinsulinemia, we found only a slight reduction in *insulin*-induced *glucose* utilization when compared with control subjects. This finding clearly indicated that *insulin* resistance in type 1 *diabetic* subjects was compensated when the concentration of *insulin* was increased. Under such conditions, the regulation of gene expression by *insulin* was found to be similar in control subjects and in type 1 *diabetic* patients. Taken together, these results strongly support the assumption that the regulation of GLUT4 gene by *insulin* is firmly associated with the responsiveness of the tissue to *insulin*.

Insulin induced a significant reduction in the mRNA levels of the two p110 catalytic subunits of PI3K in the muscle of type 2 *diabetic* patients. This result was not observed in the other groups of subjects. The consequences of this downregulation of p110(alpha)PI3K and p110(beta)PI3K ...

...require further studies to verify, at the protein and kinase activity levels, whether this regulatory mechanism may play a role in the transduction of the *insulin* signal.

The *insulin*-induced regulation of p85(alpha)PI3K, hexokinase II, and SREBP-1c mRNA expression was impaired only in type 2 *diabetic* patients, both in skeletal muscle and subcutaneous adipose tissue. Because the regulation of these three genes was normal in *insulin*-resistant nondiabetic obese subjects and in type 1 *diabetic* patients, one might thus suggest that the observed defects in type 2 *diabetes* were not secondary to *insulin* resistance, obesity, or chronic hyperglycemia. It

has been recently reported, however, that the regulation by *insulin* of hexokinase II expression in skeletal muscle was impaired not only in type 2 *diabetic* but also in nondiabetic obese subjects when low levels of *insulin* were maintained during the clamp (400-500 pmol/l) (22). Moreover, the effect of *insulin* was restored, in both groups, in the presence of very high concentrations (4,000 pmol/l) of *insulin* (22). With intermediate levels of hyperinsulinemia (1,000 pmol/l), we found that the defective regulation of hexokinase II gene expression was observed in the tissues of the type 2 *diabetic* patients specifically. Taken together, these results suggest that the concentration of *insulin* required to compensate for *insulin* resistance is an important parameter in the regulation of gene expression. However, under our experimental conditions, both the nondiabetic obese subjects and the type 2 *diabetic* patients had a similar level of *insulin* resistance as assessed by measurement of *glucose* disposal rate during the hyperinsulinemic clamp. This finding thus suggests that *insulin* resistance may not be the only cause of the defective regulation of gene expression in type 2 *diabetes*.

In addition to *insulin* resistance, a defect in the transcriptional machinery could contribute to the observed alterations. This attractive hypothesis is supported by the recent identification of mutated transcription factors in subtypes of maturity-onset *diabetes* of the young (39,40) and in other particular forms of type 2 *diabetes* (13,14). If this also occurs in the common form of type 2 *diabetes*, one call predict that the impaired regulation of gene expression may play a primary role in the pathogenesis of the disease. Recently, the transcription factor SREBP-1c has been involved in the effect of *insulin* on the transcription of several genes that encode enzymes of *glucose* and lipid metabolism (30-32). Moreover, overexpression of SREBP-1c in adipose tissue in mice is associated with *insulin* resistance, *diabetes*, and lipodystrophy (41). The promoter regions of hexokinase II and p85 (alpha)PI3K genes (E. Lefai and H. Vidal, unpublished observations) contain several SRE and...

...of its own gene (34). Therefore, SREBP-1c is a potential candidate to participate in the defective regulation of gene expression observed in type 2 *diabetes*. We have found that SREBP-1c mRNA expression is decreased in subcutaneous adipose tissue of type 2 *diabetic* obese subjects. However, this observed reduction is not likely to play a predominant role in the defective regulation of gene expression in type 2 *diabetes*. There was indeed no major alteration in the basal mRNA levels of SREBP-1c in skeletal muscle, while the defective regulation of gene expression in response to *insulin* was observed in both adipose tissue and skeletal muscle. In addition, the reduction in SREBP-1c in adipose tissue appeared to be mainly associated with obesity, and we have found that the regulation of gene expression by *insulin* was not altered in the tissues of nondiabetic obese subjects. However, it has been recently shown that, in addition to upregulating SREBP-1c gene expression (42,43), *insulin* also activates SREBP-1c transcriptional activity (43). Therefore, involvement of this transcription factor in the regulation of gene expression in human tissues and its putative role in the defective regulation observed in type 2 *diabetes* require further investigation.

The regulation of hexokinase II, p85(alpha)PI3K, and SREBP-1c gene expression is altered in skeletal muscle and adipose tissue of type 2 *diabetic* patients. These three genes could thus belong to a cluster of genes with impaired regulation by *insulin* in type 2 *diabetes*. In keeping with such a hypothesis, a common mechanism involved in their regulation, and probably of other yet unidentified genes, should be altered in the peripheral tissues of type 2 *diabetic* patients. Importantly in this context, it has been reported that *insulin* requires the PI3K pathway to control the expression of hexokinase II and p85(alpha)PI3K genes at the transcriptional level in cultured muscle cells (44,45). In hepatocyte, the same pathway is also involved in the effects of *insulin* on SREBP-1c expression and activation (43). Moreover, it has been clearly demonstrated that the activation by *insulin* of the PI3K pathway is altered in muscle of type 2 *diabetic* patients (8,9). Therefore, altered transmission of the *insulin* signal through the PI3K pathway could be involved in the impaired regulation of gene expression. However, the same pathway is also required in the actions of *insulin* oil *glucose* metabolism (4,5), and we have

shown that the response of the three genes to *insulin* was normal in tissues of *insulin*-resistant nondiabetic obese subjects. This finding suggests that the pathways involved in the regulation of gene expression and in the control of *glucose* metabolism may diverge after the activation of the PI3K and that type 2 *diabetes* may have additional defects in the pathway leading to the transcriptional regulation in the nuclei. Further works are clearly needed to decipher the mechanism of action of *insulin* from its receptor to the promoters of its target genes and to identify a common element that may be involved in the altered regulation of a cluster of genes.

In summary, *insulin* modulates in a coordinate fashion the mRNA levels of several genes involved in *insulin* action and *glucose* metabolism in skeletal muscle and in adipose tissue. We have found that the regulation of p85(alpha)PI3K, hexokinase II, and SREBP-1c gene expression by *insulin* is impaired in the tissues of type 2 *diabetic* patients. This defect appears to be independent from obesity-related *insulin* resistance and chronic hyperglycemia. These results suggest that type 2 *diabetes* may be associated with a specific alteration in the signaling to the nucleus or in the transcriptional machinery.

TABLE 1

Characteristics of the subjects

	Control...	
...6 3/6		
Age (years)	51 (+ or -) 2	43 (+ or -) 4
BMI (kg/(m.sup.2))	23 (+ or -) 1	35 (+ or -) 1 ((double dagger))
Basal		
Glucose (mmol/l)	5.0 (+ or -) 0.2	5.0 (+ or -) 0.2
Insulin (pmol/l)	37 (+ or -) 4	99 (+ or -) 17 ((double dagger))
Nonesterified fatty acid ((micro)mol/l)	399 (+ or -) 48	629 (+ or -) 49 ((dagger))
Triglycerides (mmol/l)	0.7 (+ or -) 0.1	1.2 (+ or -) 0.1 ((dagger))
Glucose disposal rate (mg * (kg.sup.-1) * (min.sup.-1))	2.2 (+ or -) 0.1	1.6 (+ or -) 0.1 ((double dagger))
Glucose oxidation rate (mg * (kg.sup.-1) * (min.sup.-1))	1.2 (+ or -) 0.0	0.9 (+ or -) 0.1
Clamp study		
Glucose (mmol/l)	4.3 (+ or -) 0.2	4.5 (+ or -) 0.1
Insulin (pmol/l)	919 (+ or -) 82	1376 (+ or -) 168
Glucose disposal rate (mg * (kg.sup.-1) * (min.sup.-1))	9.6 (+ or -) 0.9	4.5 (+ or -) 0.5 ((double dagger))
Glucose oxidation rate (mg * (kg.sup.-1) * (min.sup.-1))	3.2 (+ or -) 0.2	2.1 (+ or -) 0.1 ((double dagger))
Nonoxidative *glucose* disposal rate (mg * (kg.sup.-1) * (min.sup.-1))	6.3 (+ or -) 0.8	2.4 (+ or -) 0.4 ((double dagger))

	Type 2 *diabetes* 9	Control 25 8
Men/women	6/3	1/7
Age (years)	54 (+ or -) 3 *	25 (+ or -) 1
BMI (kg/(m.sup.2))	30 (+ or -) 1 ((double dagger)) *	22 (+ or -) 1
Basal		
Glucose (mmol/l)	10.5 (+ or -) 0.6 * ((double dagger))	4.4 (+ or -) 0.1
Insulin (pmol/l)	66 (+ or -) 10 ((double dagger))	36 (+ or -) 4

Nonesterified fatty acid ((micro)mol/l)	621 (+ or -) 67 ((dagger))	492 (+ or -) 48
Triglycerides (mmol/l)	1.4 (+ or -) 0.1 ((double dagger))	0.6 (+ or -) 0.1
Glucose disposal rate (mg * (kg.sup.-1) * (min..sup.-1))	2.4 (+ or -) 0.2 *	nd
Glucose oxidation rate (mg * (kg.sup.-1) * (min..sup.-1))	1.0 (+ or -) 0.1	1.0 (+ or -) 0.2
Clamp study		
Glucose (mmol/l)	4.9 (+ or -) 0.1	4.5 (+ or -) 0.1
Insulin (pmol/l)	1179 (+ or -) 65	875 (+ or -) 48
Glucose disposal rate (mg * (kg.sup.-1) * (min..sup.-1))	4.3 (+ or -) 0.6	10.4 (+ or -) 0.6 ((double dagger))
Glucose oxidation rate (mg * (kg.sup.-1) * (min..sup.-1))	1.9 (+ or -) 0.1	3.5 (+ or -) 0.2 ((double dagger))
Nonoxidative *glucose* disposal rate (mg * (kg.sup.-1) * (min..sup.-1))	2.5 (+ or -) 0.6	6.9 (+ or -) 0.7 ((double dagger))

	Type 1 *diabetes* 9
Men/ women	4/5
Age (years)	33 (+ or -) 3 ((dagger))
BMI (kg/(m.sup.2))	24 (+ or -) 1
Basal	
Glucose (mmol/l)	12.6 (+ or -) 0.8 ((double dagger))
Insulin (pmol/l)	--
Nonesterified fatty acid ((micro)mol/l)	519 (+ or -) 97
Triglycerides (mmol/l)	0.4 (+ or -) 0.02
Glucose disposal rate (mg * (kg.sup.-1) * (min..sup.-1))	nd
Glucose oxidation rate (mg * (kg.sup.-1) * (min..sup.-1))	1.5 (+ or -) 0.2
Clamp study	
Glucose (mmol/l)	5.6 (+ or -) 0.2 ((double dagger))
Insulin (pmol/l)	949 (+ or -) 115
Glucose disposal rate (mg * (kg.sup.-1) * (min..sup.-1))	8.6 (+ or -) 0.8
Glucose oxidation rate (mg * (kg.sup.-1) * (min..sup.-1))	3.4 (+ or -) 0.2
Nonoxidative *glucose* disposal rate (mg * (kg.sup.-1) * (min..sup.-1))	5.3 (+ or -) 0.8

((dagger)) P < 0.05 and

((double dagger)) P < 0.01 vs. the respective control subjects;

* P < 0.05 between type 2 *diabetic* and obese subjects; nd, not determined.

TABLE 2

Relative effects of *insulin* infusion on the expression of the target genes in skeletal muscle

	Control 50	Obese
Insulin receptor	36 (+ or -) 20	-8 (+ or -) 12
IRS-1	-47 (+ or -) 6 *	-31 (+ or -) 6 *
p85(alpha)PI3K	93 (+ or -) 20 *	78 (+ or -) 18 *
p110...		

...Hexokinase II	93 (+ or -) 20 *	127 (+ or -) 35 *
Rad	223 (+ or -) 68 *	112 (+ or -) 34 *
Glycogen synthase	32 (+ or -) 29	-2 (+ or -) 21

	Type 2 *diabetes*	Control 25
Insulin receptor	12 (+ or -) 20	20 (+ or -) 12
IRS-1	-27 (+ or -) 9 *	-34 (+ or -) 4 *
p85(alpha)PI3K	-1 (+ or -) 12 ((dagger))	128 (+ or -) 46...

...II	9 (+ or -) 18 ((dagger))	194 (+ or -) 37 *
Rad	60 (+ or -) 22 *	190 (+ or -) 37 *
Glycogen synthase	19 (+ or -) 13	15 (+ or -) 9

	Type 1 *diabetes*
Insulin receptor	32 (+ or -) 18
IRS-1	-31 (+ or -) 9 *
p85(alpha)PI3K	67 (+ or -) 20 *
p110(alpha)PI3K	-5 (+ or -) 15
p110(beta)PI3K	47...

...clamp and the basal values.

* Significant change (P < 0.05 with Wicoxon's nonparametric test for paired values) when comparing the values before and after *insulin* infusion.

((dagger)) Significant difference in the effect of *insulin* in a group of patients when compared with the age-matched control group.

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Pierre-Henri...

..la Recherche Medicale (INSERM) U.449 and (2) Human Nutrition Research Center of Lyon, Faculty of Medicine R. Laennec; and the (3) Department of Endocrinology, *Diabetology*, and Nutrition, E. Herriot Hospital, Lyon, France.
Address correspondence and reprint requests to Hubert Vidal, PhD, INSERM U.449, Faculte de Medecine R. Laennec, Rue...

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P.-H.D. and N.P. contributed equally to this work.
IRS, *insulin* receptor substrate; PCR, polymerase chain reaction; PI3K, phosphatidylinositol 3-kinase; p85(alpha)*PI3K*, *p85*(alpha) regulatory subunit of phosphatidylinositol 3-kinase; p110(alpha)PI3K, p110(alpha) catalytic subunit of phosphatidylinositol 3-kinase; p110(beta)PI3K, p110(beta) catalytic subunit of phosphatidylinositol 3-kinase; Rad, Ras protein associated with *diabetes*; RT-cPCR, reverse transcriptase-competitive polymerase chain reaction; SREBP-1c, sterol regulatory-element-binding protein-1c.

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